

BIONETICS

MUTAGENIC EVALUATION OF COMPOUND 025013165

BUTYLATED HYDROXYANISOLE

(SUSTANE) FDA 71-24

7315 Wisconsin Avenue Bethesda, Maryland 20014

LBI PROJECT #2468

MUTAGENIC EVALUATION OF

COMPOUND 025013165

BUTYLATED HYDROXYANISOLE

(SUSTANE) FDA 71-24

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC. 5516 NICHOLSON LANE KENSINGTON, MARYLAND

APRIL 30, 1975



TABLE OF CONTENTS

EVALUATION SUMMARY	Page No.
I. OBJECTIVE	2
II. <u>MATERIALS</u>	2
A. Test Compound B. Indicator Microorganisms C. Reaction Mixture D. Tissue Homogenates and Supernatants E. Positive Control Compounds	
III. METHODS	3
A. Toxicity B. Plate Tests C. Suspension Tests D. Preparation of Tissue Homogenates and 9,000 Fractions E. Data Recording and Reporting	
IV. RESULTS SECTION	
A. Solubility Properties of the Test Compound B. Toxicity and Dosage Determinations for the 1 Compound	Test
V. <u>SUMMARY OF TEST RESULTS</u>	7
VI. INTERPRETATION OF RESULTS AND CONCLUSIONS	14
A. Salmonella typhimurium. B. Saccharomyces cerevisiae. C. Conclusions.	14
APPENDIX-TABULATION OF DATA	Δ_1



EVALUATION SUMMARY

Compound FDA 71-24, Butylated Hydroxyanisole, did not exhibit genetic activity in any of the $\underline{\text{in}}$ $\underline{\text{vitro}}$ microbial assays employed in this evaluation.

DATE: April 30, 1975

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound 025013165, Butylated Hydroxyanisole

(Sustane)

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

- A. <u>Test Compound</u>
- 1. Date Received: August, 1974
- Description: Beige Crystalline Material
- B. <u>Indicator Microorganisms</u>

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: <u>Salmonella</u> <u>typhimurium</u>, strains:TA-1535 TA-1537

TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

	Component	Final Concentration/ml
2. 3. 4.	TPN (sodium salt) Isocitric acid Tris buffer, pH 7.4 MgCl ₂ Tissue homogenate fraction	6 μ M 49 μ M 28 μ M 1.7 μ M 72 mg



D. <u>Tissue Homogenates and Supernatant</u>

The tissue homogenates and 9,000 x \underline{g} supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

E. <u>Positive Control Compounds</u>

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1 POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical ^a	Solvent	Probable Mutagenic Specificity
Non-activation	Ethyl methanesulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide ^C	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide ^C	FS

Concentrations given in the Results Section
BPS = base-pair substitution; FS = frameshift
Previously shown to be non-mutagenic

III. METHODS

A. <u>Toxicity</u>

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



B. Plate Tests

In the nonactivation procedure, approximately 10° cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

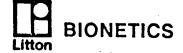
C. Suspension Tests

Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1 x 10^9 cells/ml and 5 x 10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days' before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.



D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in icecold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C . The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C . These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.



- IV. RESULTS SECTION
- A. Solubility Properties of the Test Compound
- Name or code designation of the test compound:
 025013165 Butylated Hydroxyanisole (Sustane)
- 2. Test solvent: DMSO
- 3. Solubility of the test compound under treatment conditions: Soluble under treatment conditions.
- 4. Additional comments: beige crystalline material
- B. <u>Toxicity and Dosage Determinations for the Test Compound</u>
- 1. Test date for toxicity determination: November 13, 1974
- 2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0

0.5

0.05

0.005

0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

Dose Bacteria Yeast 1/4 50% Survival 0.00375 0.0625 1/2 50% Survival 0.0075 0.1250 50% Survival 0.0150 0.02500 Plate Tests 0.0075 -



IV. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: 025013165

B. Test date: April 23, 1975

C. Concentration of the test compound: 0.0075%

Test		Species	Tiss	sue	TA	<u>-1535</u>	<u>TA</u>	<u>-1537</u>	TA	-1538
1.	Non-activation				1	2	1	2	1	2
	Solvent Control Positive Control ^a Test Compound		~	•	138 >10 ⁴ 207	146 >10 ⁴ 226	25 193 13	22 176 19	26 158 34	35 219 32
2.	Activation									
	Negative Control Solvent Control Reaction Mixture				16 12	15 9	27 36	23 43	11 17	14 18
	Control			ı	10	18	36	39	9	17
	Positive Control Positive Control Positive Control	Mouse	Live Lung Test		>10 ³ 9 11	>10 ³ 8 7	146 33 37	143 33 32	239 14 15	225 11 15
	Positive Control Positive Control Positive Control	Rat	Live Lung Test		>10 ³ 10 11	>10 ³ 8 6	84 32 24	80 35 43	329 16 16	313 12 16
	Positive Control Positive Control Positive Control	Monkey	Live Lung Test		>10 ³ 7 9	>10 ³ 9 5	47 32 28	43 38 33	122 15 15	129 10 15
	Test Compound Test Compound Test Compound	Mouse	Live Lung Test		8 11 10	15 8 8	40 28 38	47 33 44	12 5 16	9 11 8
	Test Compound Test Compound Test Compound	Rat	Live Lung Test		8. 13 13	11 10 8	46 28 37	44 32 46	16 5 15	12 11 9
•	Test Compound Test Compound Test Compound	Monkey	Live Lung Test		6 12 15	11 10 9	38 38 35	45 33 36	20 5 14	12 10 12
•	TA-1537 QM 20 μ	l/plate g/plate g/plate	b	TA-1 TA-1 TA-1	537	DMNA AAF AAF	100 μ	M/plate g/plate g/plate	•	



DATA TABLE TERMS AND ABBREVIATIONS

OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s)
	A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound A+T = Activation: Tissue Control
	LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.
	Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + $6 = X \cdot 10^6$).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = \times 100). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION					
AAF	2-Acetylaminofluorene					
DMSO	Dimethylsulfoxide					
DMN	Dimethylnitrosamine					
EMS	Ethyl Methanesulfonate					
QM	Quinacrine Mustard					
NF	Nitrofluorene					
SPECIES	Animal Strains					
SPRDAW	Sprague Dawley Rats					
ICRFLO	Flow ICR Random Bred Mice					
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)					
MIXEDB	Dog, Mixed Breed					
NEWZEA	New Zealand White Rabbit					



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES

COMPOUND 025013165

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
NAN		1.22	4.28	9.79	3.83	58.58
NAP		1088.21	415.68	181.23	39.75	172.46
NA1		2.37	2.45	6.07	2.44	54.50
NA2		2.42	3.60	8.79	5.40	57.39



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES ICRFLO COMPOUND 025013165

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	2.63	3.70	4.95	3.56	4.07	
ACT	A-C	1.05	3.44	2.56	3.82	6.21	
ACT	PL I	58.39	14.57	36.50	7.31	9•96	
ACT	PLU	1.15	3.31	4.31	3.71	3.84	
ACT	PTE	9.55	3.74	3.35	3.06	6.12	
ACT	LII	2.96	3.02	2.05	3.63	4.32	
ACT	LI2	2.16	4.47	2.42	4.41	4.51	
ACT	LU1	0.69	2.77	3.49	2.98	3.65	
ACT	LU2	0.91	2.90	3.13	4.74	7.01	
ACT	TE1	3.71	3.34	3.84	2.82	4.34	
ACT	TE2	2.33	3.39	4.12	4.03	5.20	



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES SPRDAW

COMPOUND 025013165

TEST _.	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	3.12	10.83	10.78	2.69	2.22
ACT	A-C	4.26	8.33	5.64	4.67	3.00
ACT	PL I	49.05	18.14	129.82	6.49	4.25
ACT	PLU	2.70	5.39	15.38	4.51	4.26
ACT	PTE	3.52	12.52	6.04	2.91	4.07
ACT	LII	4.12	5.95	3.64	2.22	0.62
ACT	LI2	2.88	6.57	3.47	1.86	0.88
ACT	LU1	0.93	6.97	1.57	3.84	5.25
ACT	LU2	2.84	6.44	1.92	0.74	1.47
ACT	TE1	4.05	7.70	5.31	0.97	0.00
ACT	TE2	5.75	6.75	7.59	3.29	0.66



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 06/05/75

		(SPECIES RHESUS		CUMPOUSD 02501310		
TEST	ORG		TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADF FX-5	TRY	
ΛCΤ	Δ+C	5.36	5.04	15.13	1.27	1.63	
ACT	Δ+Τ				1.55	1.74	
ACT	∨ −C	8.28	3.26	4.81	2.45	1.57	
ACT	bŗ I	197.63	14.44	49,46	5.95	6.31	
ACT	PLU	15.17	2.95	4.60	1.53	1.53	
ΔΟΤ	PTE	5.03	5.26	5.69	2.12	1.34	
ACT	LI1	2.29	4.26	1.15	2.17	2.02	
ACT	L12	5.43	2.25	7.30	1.43	1.56	
ΔCΤ	· LU1	2.78	3.09	7.04	1.96	1.68	
ACT	1.02	7.18	1.69	14.20	1.69	1.24	
ΔCT	TF1	1.92	4.86	4 _• 05	3.13	1.09	
AC T	TE2	3.76	3.36	10.04	2.68	1.55	

10.04



V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound FDA 71-24, Butylated Hydroxyanisole, was evaluated for genetic activity in a series of <u>in vitro</u> microbial assays with and without metabolic activation. The following results were obtained:

- A. <u>Salmonella typhimurium</u>
- 1. Plate tests

At a concentration of 0.0075%, this compound was not mutagenic for any of the bacteria indicator strains in direct or activation plate tests.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

- B. <u>Saccharomyces cerevisiae</u>
- 1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. <u>Conclusions</u>

The test compound, Butylated Hydroxyanisole, did not exhibit genetic activity in any of the assays employed in this evaluation.

Submitted by:

David Brusick, Ph.D. Director of Genetics

APPENDIX

Tabulation of Data





CONTRACT EXPERIMENT 431701			22374-2104 DETECTOR TA1535	SPE	CIES	PROJECT 02468 DATE - 04/25/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	0576	0007	1.22	2
	NAP		EMS 0.002 %	0390	4244	1088.21	0
025013165	NA1		0075-4 PCT.	0253	0006	2.37	0
025013165	NAZ		0375-5 PCT.	0372	0009	2.42	o



CONTRACT			TRACT	22374-2104			PROJECT 02468	
EXPERIMENT 431702		DETECTOR TA1537 SPECIE		CIES	IES DATE - 04/25/75			
			ORG		POPU	MUT1	FREQ1	
	COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM
		NAN		SALINE	0817	0035	4.28	0
		NAP		QM 1.0 UG/ML	0236	0981	415.68	0
	025013165	NAI		0075-4 PCT.	1022	0025	2.45	0
	025013165	NA2		0375-5 PCT.	1193	0043	3.60	0



CONTRACT 22374-2104						PROJECT 02468	
EXPERIMENT	T 4317	03	DETECTOR TA1538 SPE		CIES	DATE -	04/25/75
	•	ORG	·	POPU	MUT1	FREQ1	
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM
	NAÑ		DMSO	0337	0033	9.79	0
	NAP		NF 125 UG-ML	0341	0618	181.23	0 .
025013165	NA1		0075-4 PCT.	0280	0017	6.07	0
025013165	NA2		0375-5 PCT.	0398	0035	8.79	0



CONTRACT 22374-2104						PRO.	JECT 024	58	÷
EXPERIMEN	T 5016	02	DETECTOR 0000D4	4 SPE	SPECIES DATE - 04/25/75				
		ORG		POPU	MUT1	MUT2	FREQ1	FRE02	
COMPOUND	TEST	ΙD	CONCENTRATION	EP+4	EP+1	EP+1	FP-5	EP-5	CONTAM
025013165	NA1		0075-4 PCT.	0655	0016	0357	2.44	54.50	0
025013165	NA2		0375-5 PCT.	0352	0019	0202	5.40	57.39	0
	NAN		SALINE	0548	0021	0321	3.83	58.58	0
	NAP	EMS	11.0 PCT	0483	0192	0833	39.75	172.46	0



		22374-2104			PROJECT 02468			
EXPERIMEN.	T 4323	01	DETECTOR TA1535	SPE	CIES ICR	FLO DATE -	04/25/75	
		ORG		POPU	MUT1	FREQ1		
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM	
	A+C		DMN 50 UM/ML	0799	0021	2.63	0	
	A- C		SALINE	0667	0007	1.05	0	
	ACP	LI	DMN 50 UM/ML	0793	0463	58.39	0	
	ACP	LU	DMN 50 UM/ML	0695	8000	1.15	2	
	ACP	TE	DMN 50 UM/ML	0555	0053	9•55	2	
025013165	ACT	LII	0075-4 PCT.	0777	0023	2.96	• 0	
025013165	ACT	LI2	0375-5 PCT.	0879	0019	2.16	2	
025013165	ACT	LU1	0075-4 PCT.	0434	0003	0.69	2	
025013165	ACT	LU2	0375-5 PCT.	0438	0004	0.91	0	
025013165	ACT	TE1	0075-4 PCT.	0673	0025	3.71 .	2	
025013165	ACT	TE2	0375-5 PCT.	0774	0018	2.33	2	



EVDED IMEN'			22374-2104 DETECTOR TA1537	CDE	רזבכ זרם	PROJECT 0		04/25/75
EXPERIMEN.	1 4524	01	DETECTOR TAIDS?	SPE	CIES ICK	FLU	DATE -	04/25/15
		ORG		POPU	MUT1	• FREQ	1	
COMPOUND	TEST	ΙD	CONCENTRATION	EP+6	EP+0	EP-8		CONTAM
	A+C		AAF 800 UG/ML	1677	0062	3.7	0	0
S.	A-C		DMSO	1365	0047	3•.4	4	0
	ACP	LI	AAF 800 UG/ML	1119	0163	14.5	7	0
	ACP	LU	AAF 800 UG/ML	1180	0039	3.3	1	2
	ACP	TE	AAF 800 UG/ML	1417	0053	3.7	4	2
025013165	ACT	LII	0075-4 PCT.	1225	0037	3.0	2	2
025013165	ACT	LI2	0375-5 PCT.	1119	0050	4 • 4	7	2
025013165	AC T	LU1	0075-4 PCT.	0723	0020	2.7	7	2
025013165	ACT	LU2	0375-5 PCT.	0863	0025	2.9	0	2
025013165	ACT	TE1	0075-4 PCT.	1257	0042	3.3	4	2
025013165	ACT	TE2	0375-5 PCT.	1621	0055	3.3	9 .	2



CONTRACT			22374-2104		PROJECT 02468				
EXPERIMEN'	T 4325	01	DETECTOR TA1538	SPE	CIES ICR	FLO DATE -	04/25/75		
		ORG		POPU	MUT1	FREQ1	CONTAM		
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM		
	A+C		AAF 800 UG/ML	0646	0032	4.95	0		
	A-C		DMSO	0742	0019	2.56	0		
•	ACP	LI	AAF 800 UG/ML	0737	0269	36.50	2		
	ACP	LU	AAF 800 UG/ML	0510	0022	4.31	0		
	ACP	TE	AAF 800 UG/ML	0717	0024	3.35	2		
025013165	ACT	LII	0075-4 PCT.	0782	0016	2.05	2		
025013165	ACT	LI2	0375-5 PCT.	0454	0011	2.42	2		
025013165	ACT	LU1	0075-4 PCT.	0258	0009	3.49	0		
025013165	ACT	LU2	0375-5 PCT.	0351	0011	3.13	2		
025013165	ACT	TE1	0075-4 PCT.	0704	0027	3.84	2		
025013165	ACT	TE2	0375-5 PCT.	0510	0021	4.12	2		



	22374-21	04	PROJECT 02468							
EXPERIMENT		TRACT	DETECTOR	000004	SPE	CIES I	CRFLO	DATE - 04/25/75		
COMPOUND	TEST	ORG ID	CONCENTR	RATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 L	JM/ML	0787	0028	0032	3.56	4.07	6
	A-C		SALINE	,	0837	0032	0052	3.82	6.21	0
	ACP	LI	DMN 90 L	JM/ML	0793	0058	0079	7.31	9.96	0
	ACP	LU	DMN 90 U	IM/ML	0781	0029	0030	3.71	3.84	0
	ACP	TE	DMN 90 L	JM/ML	0556	0017	0034	3.06	6.12	6
025013165	ACT	LII	0075-4 P	CT.	1019	0037	0044	3.63	4.32	7
025013165	ACT	LI2	0375-5 P	CT.	1065	0047	0048.	4.41	4.51	7
025013165	ACT	LU1	0075-4 P	CT.	1041	0031	0038	2.98	3.65	3
025013165	ACT	LU2	0375-5 P	PCT.	0970	0046	0068	4.74	7.01	7
025013165	ACT	TE1	0075-4 P	PCT•	0921	0026	0040	2.82	4.34	0
025013165	ACT	TE2	0375-5 P	PCT.	0943	0038	0049	4.03	5.20	7



CONTRACT EXPERIMENT 431801		ITRACT	22374-2104			PROJECT 02468	
EXPERIMEN'	T 4318	01	DETECTOR TA1535	SPE	CIES SF	PRDAW DATE - (04/25/75
COMPOUND	***	ORG	60005017047760	POPU	MUT1	FRE01	
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM
	A+C		DMN 50 UM/ML	0513	0016	3.12	0
	A-C		SALINE	0470	0020	4.26	0
	ACP	LI	DMN 50 UM/ML	0422	0207	49.05	0
	ACP	LU	DMN 50 UM/ML	0370	0010	2.70	0
·	ACP	TE	DMN 50 UM/ML	0227	8000	3.52	0
025013165	ACT	LII	0075-4 PCT.	0194	8000	4.12	2
025013165	ACT	LI2	0375-5 PCT.	0278	0008	2.88	0
025013165	ACT	LU1	0075-4 PCT.	0108	0001	0.93	0
025013165	ACT	LU2	0375-5 PCT.	0282	0008	2.84	0
025013165	ACT	TE1	0075-4 PCT.	0148	0006	4.05	0
025013165	ACT	TE2	0375-5 PCT.	0226	0013	5.75	0



			22374-2104	PROJECT 02468				
EXPERIMEN"	T 4319	901	DETECTOR TA1537	SPE	SPECIES SPRDAW		04/25/75	
		ORG		POPU	MUT1	FREQ1		
COMPOUND	TEST	10	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM	
	A+C		AAF 800 UG/ML	0600	0065	10.83	0	
	A-C		DMSO	0840	0070	8.33	0	
	ACP	LI	AAF 800 UG/ML	0998	0181	18.14	0	
	ACP	LU	AAF 800 UG/ML	1169	0063	5.39	0 .	
	ACP	TE	AAF 800 UG/ML	0735	0092	12.52	0	
025013165	AC T	LII	0075-4 PCT.	0975	0058	5.95	2	
025013165	ACT	LI2	0375-5 PCT.	1188	0078	6.57	0	
025013165	AC T	LU1	0075-4 PCT.	0674	0047	6.97	0	
025013165	ACT	LU2	0375-5 PCT.	0839	0054	6.44	0	
025013165	ACT	TE1	0075-4 PCT.	0844	0065	7.70	o	
025013165	ACT	TE2	0375-5 PCT.	0874	0059	6.75	o	



CONTRACT EXPERIMENT 432201			22374-2104 DETECTOR TA1538	PROJECT 02468 SPECIES SPRDAW DATE - 04/25/75				
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM	
	A+C	• "	AAF 800 UG/ML	0371	0040	10.78	0	
	A-C		DMSO	0443	0025	5.64	0	
•	ACP	LI	AAF 800 UG/ML	0228	0296	129.82	0	
	ACP	LU	AAF 800 UG/ML	0312	0048	15.38	0	
	ACP	TE	AAF 800 UG/ML	0298	0018	6.04	0	
025013165	ACT	LII	0075-4 PCT.	0302	0011	3.64	2	
025013165	ACT	LI2	0375-5 PCT.	0461	0016	3.47	. 2	
025013165	ACT	LU1	0075-4 PCT.	0255	0004	1.57	0	
025013165	ACT	LU2	0375-5 PCT.	0260	0005	1.92	0	
025013165	ACT	TE1	0075-4 PCT.	0207	0011	5.31	0	
025013165	ACT	TE2	0375-5 PCT.	0237	0018	7.59	0	

		TRACT	22374-2104				JECT 0246	CT 02468		
EXPERIMENT	T.4343	301	DETECTOR 0000D4	SPE	CIES S	SPRDAW	DA	TE - 04/	25/75	
COMPOUND	TEST	ORG I D	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM	
	A+C		DMN 90 UM/ML	0854	0023	0019	2.69	2.22	, 0	
	A-C		SALINE	1135	0053	0034	4.67	3.00	0	
	ACP	LI	DMN 90 UM/ML	0848	0055	0036	6.49	4.25	0	
	ACP	LU	DMN 90 UM/ML	0821	0037	0035	4.51	4.26	0	
	ACP	TE	DMN 90 UM/ML	0860	0025	0035	2.91	4.07	0	
025013165	ACT	LII	0075-4 PCT.	0811	0018	0005	2.22	0.62	, 6	
025013165	ACT	LI2	0375-5 PCT.	1023	0019	0009	1.86	0.88	6	
025013165	ACT	LU1	0075-4 PCT.	0781	0030	0041	3.84	5.25	-6	
025013165	ACT	LU2	0375-5 PCT.	0950	0007	0014	0.74	1.47	7	
025013165	ACT	TE1	0075-4 PCT.	0619	0006	0000	0.97	0.00	7	
025013165	ACT.	TE2	0375-5 PCT.	0759	0025	0005	3.29	0.66	7	

EXPERIMENT 432604			22374-2104 DETECTOR TA1535	PROJECT 02468 SPECIES RHESUS DATE - 04/25/75				
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM	
	A+C		DMN 50 UM/ML	0392	0021	5.36	0	
	A-C		SALINE	0338	0028	8.28	0	
	ACP	LI	DMN 50 UM/ML	0337	0666	197.63	0	
•	ACP	LU	DMN 50 UM/ML	0211	0032	15.17	0	
	ACP	TE	DMN 50 UM/ML	0298	0015	5.03	0	
025013165	ACT	LII	0075-4 PCT.	0262	0006	2•29	2	
025013165	ACT	LI2	0375-5 PCT.	0368	0020	5•43	2	
025013165	ACT	LUI	0075-4 PCT.	0288	8000	2.78	. 0	
025013165	ACT	LU2	0375-5 PCT.	0376	0027	7.18	2	
025013165	ACT	TE1	0075-4 PCT.	0052	0001	1.92	0	
025013165	ACT	TE2	0375-5 PCT.	0213	0008	3.76	0	



CONTRAC EXPERIMENT 433101			22374-2104 DETECTOR TA1537	SPE	CIES RHES	PROJECT 02468 RHESUS DATE - 04/25/75		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 FP-8	CONTAM	
	A+C		AAF 800 UG/ML	0794	0040	5.04	0	
	A-C		DMSO	1135	0037	3.26	0	
	ACP	ŁI	AAF 800 UG/ML	1122	0162	14.44	0	
	ACP	LU	AAF 800 UG/ML	0848	0025	2.95	0	
	ACP	TE	AAF 800 UG/ML	0874	0046	5•26	0	
025013165	AC T	LII	0075-4 PCT.	0540	0023	4.26	O	
025013165	ACT	LI2	0375-5 PCT.	1066	0024	2•25	0	
025013165	ACT	LU1	0075-4 PCT.	0713	0022	3.09	0	
025013165	ACT	LU2	0375-5 PCT.	1005	0017	1.69	0	
025013165	ACT	TE1	0075-4 PCT.	0535	0026	4.86	0	
025013165	· ACT	TE2	0375-5 PCT.	0803	0027	3.36	0	



CONTRACT EXPERIMENT 433601			22374-2104	PROJECT 02468					
			DETECTOR TA1538	SPE	CIES RHESU	DATE -	ATE - 04/25/75		
		ORG		POPU	MUT1	FREQ1			
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8	CONTAM		
	A+C		AAF 800 UG/ML	0152	0023	15.13	0		
	A-C		DMSO	0187	0009	4.81	0		
	ACP	LI	AAF 800 UG/ML	0279	0138	49.46	0		
	ACP	LU	AAF 800 UG/ML	0261	0012	4.60	1		
	ACP	TE	AAF 800 UG/ML	0246	0014	5.69	0		
025013165	ACT	LI1	0075-4 PCT.	0434	0005	1.15	2		
025013165	ACT	LI2	0375-5 PCT.	0562	0041	7.30	0		
025013165	AC T	LU1	0075-4 PCT.	0426	0030	7.04	0		
025013165	ACT	LU2	0375-5 PCT.	0521	0074	14.20	0		
025013165	ACT	TE1	0075-4 PCT.	0444	0018	4.05	0		
025013165	ACT	TE2	0375-5 PCT.	0458	0046	10.04	0		



CONTRACT EXPERIMENT 514302			22374-2104 DETECTOR 000004	PROJECT 02468 SPECIES RHESUS DATE - 06/05/75					
		ORG		POPU	MHT1	MUT2	FREGI	ERFO2	
COMBOHNO	TFST	ΙD	CONCENTRATION	EP+4	EP+1	FP+1	FP-5	FP-5	CONTAM
	Λ+C		DMN 90 UH/ML	1102	0014	0018	1.27	1.63	4
	Λ + Ţ		***NO MV LCH***	0516	0008	0009	1.55	1.74	n
	A-C		SALINE	1020	0025	0016	2.45	1.57	1
	VCP	LI	DMN 90 UM/ML .	0555	0033	0035	5.95	6.31	0
	ACP	LU	DMM 90 UM/ML	0783	0012	0012	1.53	1.53	0
	ACP	TE	DWN 90 UM/ML	0896	0019	0012	2.12	1.34	0
025013165	ACT	1.11	0075-4 PCT.	0645	0014	0013	2.17	2.02	0
025013165	ΛCΤ	L I 2	0375-5 PCT.	0767	0011	0012	1.43	1.56	0
025013165	ACT	LUL	0075-4 PCT.	0713	0014	0012	1.96	1.68	0
025013165	ACT	LUZ	0375-5 PCT.	0890	0015	0011	1.69	1.24	1
025013165	ACT	TE1	0075-4 PCT.	0,640	0020	0007	3.13	1.09	n
025013165	ACT	TF2	0375-5 PCT.	0710	0019	0011	2.68	1.55	Ο